Towards integrated models of regulatory networks: the MetaGenoReg project

Hidde de Jong and Daniel Kahn

INRIA Grenoble - Rhône-Alpes

Hidde.de-Jong@inria.fr

http://ibis.inrialpes.fr

LBBE, Université de Lyon

Daniel.Kahn@univ-lyon1.fr

http://lbbe.univ-lyon1.fr

LBBE

INSTITUT NATIONAL DE RECHERCHE EN INFORMATIQUE ET EN AUTOMATIQUE | INRIA | centre de recherche GRENOBLE - RHÔNE-ALPES

Lyon 1

INRA
Institut National de la Recherche Agronomique

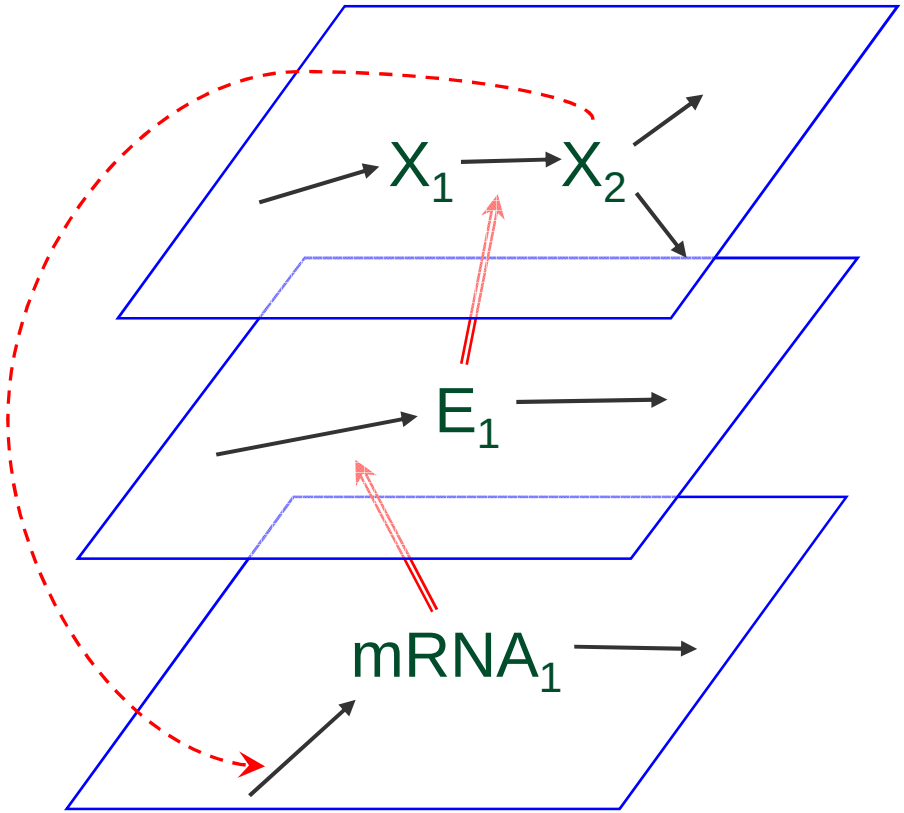
Overview

1. General question of biological regulation:
   the MetaGenoReg project
2. Biological system: the glucose-acetate diauxie in *E. coli*
3. Methodological approach
4. Metabolic model
5. Integrating gene and metabolic models

1. General question of biological regulation

- Cellular regulation involves several levels, including:
  - Gene regulatory networks
  - Metabolic regulation
- These levels interact:
  - Gene expression impacts metabolism through changes in enzyme concentrations
  - Conversely metabolism influences gene expression
- What is the rationale articulating both types of regulation?
  - Are they interchangeable ?
  - How much are they constrained?
  - What is the relative importance of gene and metabolic regulation?

‘Hierarchical’ analysis



module 1
metabolism

module 2
protein level

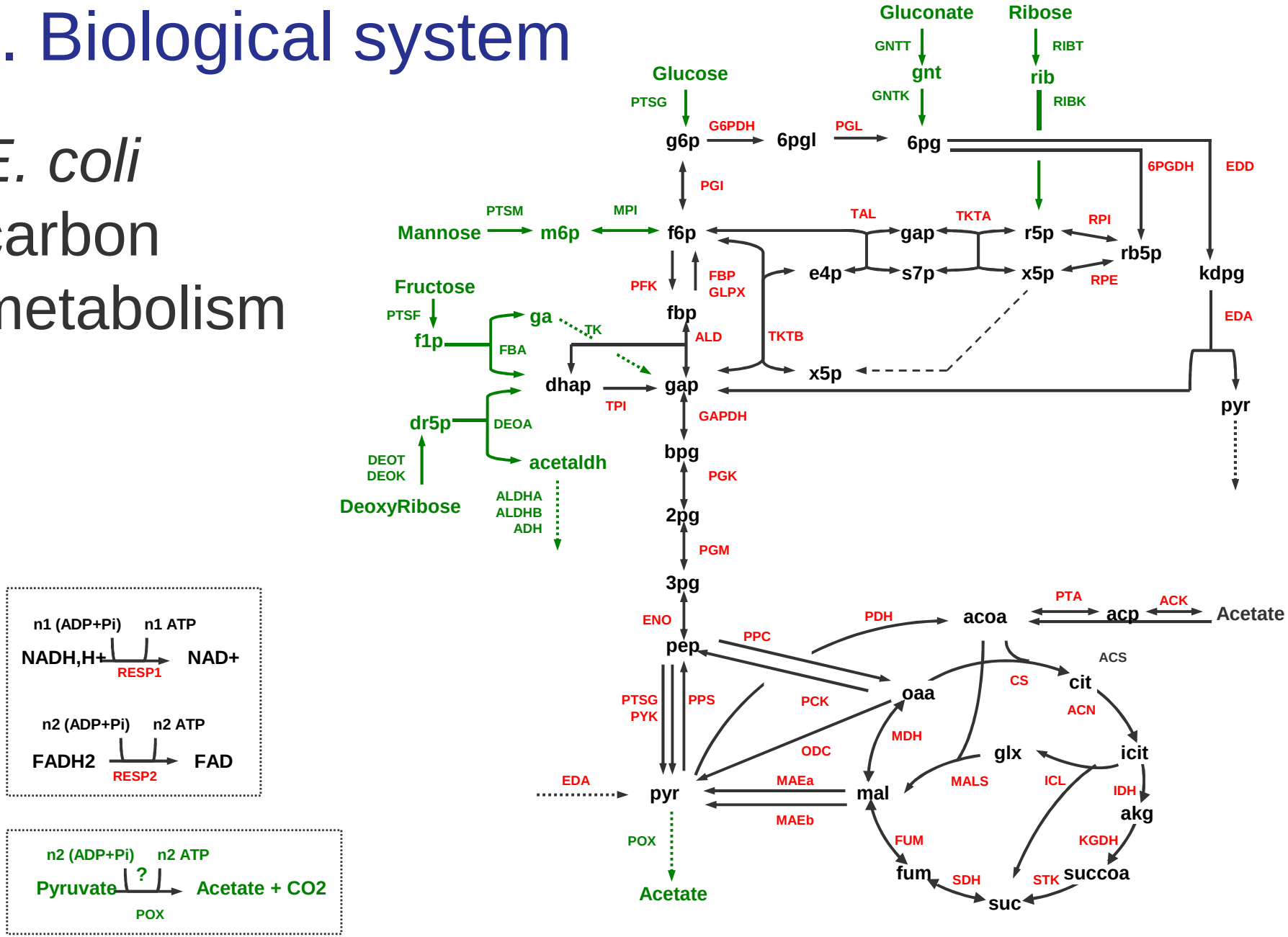
module 3
RNA level

MetaGenoReg project outline

- Modelling combined metabolic and gene regulation
  - Reduce and simplify in order to understand the system's behaviour
  - Develop a method for joint modelling combining different approximations suited to both types of regulation
  - Measure their respective contribution
- Analyse the model's strengths and weaknesses from a systemic point of view
- Understand the biological rationale underlying the distribution of regulation between metabolism and gene expression

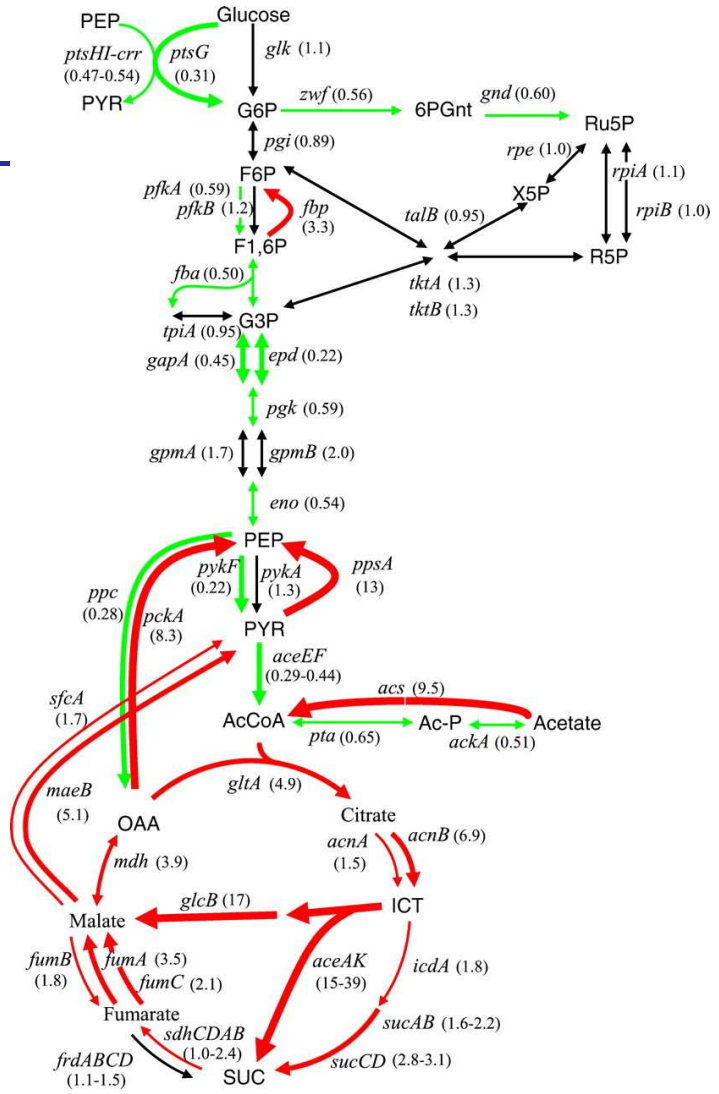
2. Biological system

E. coli carbon metabolism



Glucose-acetate diauxie

- Well-characterised transition in *E. coli*
- Involves major changes
  - at the metabolic level: gluconeogenesis *vs.* glycolysis
  - at the gene expression level
- Strong interaction between metabolic and gene expression levels

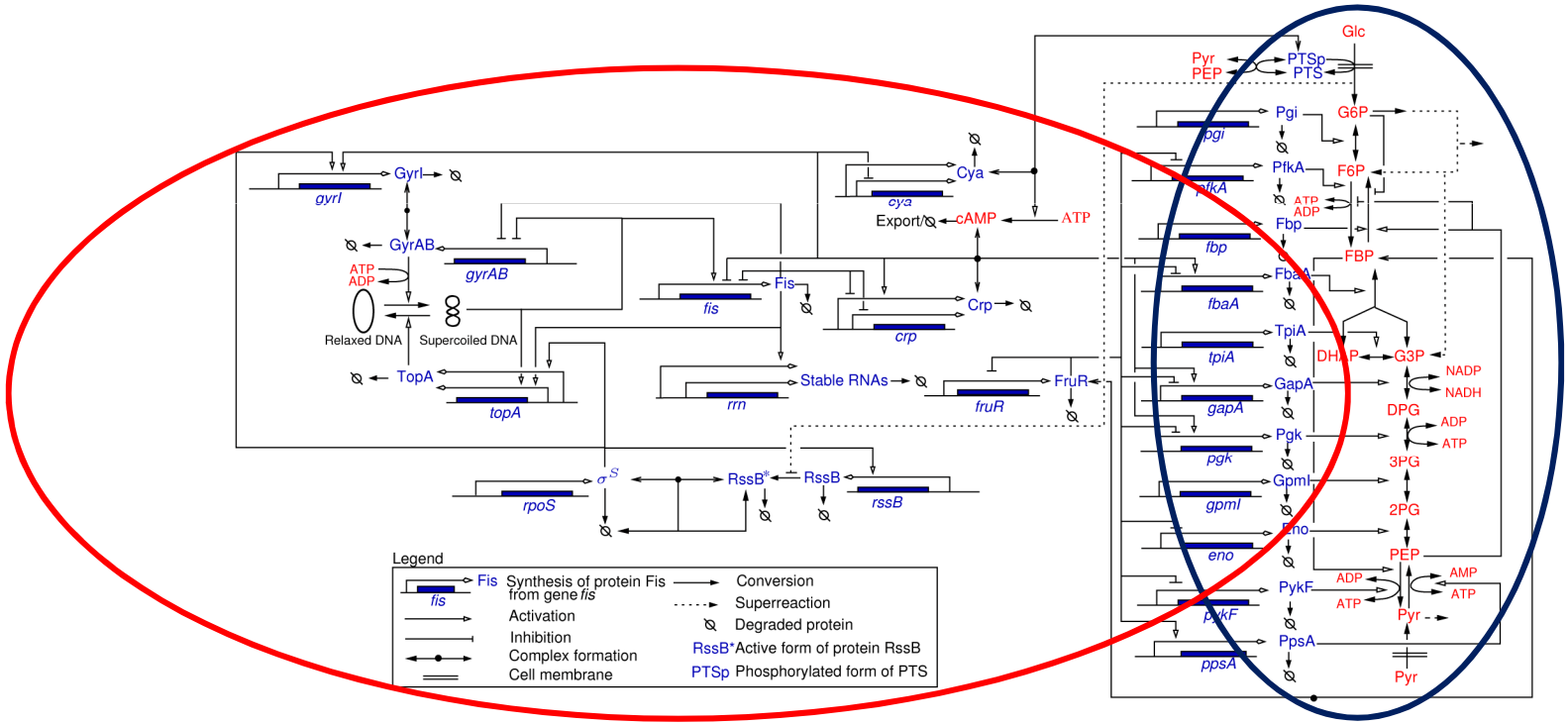


Oh *et al.* (2002), *J Biol Chem.* 277(15):13175-83.

Carbon assimilation in *E. coli*

- Interactions between metabolism and gene expression involve complex regulatory networks

  Genetic and metabolic control of glycolysis and gluconeogenesis



Baldazzi *et al.* (2010), *PLoS Comput. Biol.*, 6(6):e1000812

3. Methodological approach

- Development of integrated model of upper-part of carbon assimilation in *E. coli*
- Kinetic model consisting of 41 variables and more than 100 parameters
- Main problems with model: lack of parameter values, lack of data to estimate parameter values
- **Basic assumption for model reduction**: on the time-scale of gene expression, metabolism is a fast process

  In bacteria, time constants for gene expression are typically of the order of (tens of) minutes, whereas time constants in metabolism are typically of the order of seconds

Model reduction using time-scale hierarchy

- How is time-hierarchy exploited to formally reduce kinetic model of integrated genetic and metabolic network?
- Basic form of kinetic model $\dot{x} = N\, v(x)$
  - Concentration variables $x \in \mathbb{R}^n_+$
  - Reaction rates $v : \mathbb{R}^n_+ \longrightarrow \mathbb{R}^q$
  - Stoichiometric matrix $N \in \mathbb{Z}^{n \times q}$
- Time-scale hierarchy motivates distinction between **fast** reaction rates $v^f \in \mathbb{R}^{q-p}$ and **slow** reaction rates $v^s \in \mathbb{R}^p$ such that

$$v = [v^s \;\; v^f]'$$

Typically, enzymatic and complex formation reactions are fast, protein synthesis and degradation are slow

Model reduction using time-scale hierarchy

- Separation of fast and slow reactions motivates a linear transformation $T \in \mathbb{Z}^n \times \mathbb{Z}^n$ of the variables

$$\begin{bmatrix} x^s \\ x^f \end{bmatrix} = T\,x \quad \text{such that} \quad \begin{bmatrix} N^s & 0 \\ N^{s'} & N^f \end{bmatrix} = T\,N$$

- We call $x^s \in \mathbb{R}_+^m$ **slow variables** and $x^f \in \mathbb{R}_+^{n-m}$ **fast variables**, while $N^s \in \mathbb{Z}^m \times \mathbb{Z}^p$ and $N^{s'} \in \mathbb{Z}^{n-m} \times \mathbb{Z}^p$ are stoichiometric matrices for slow reactions and $N^f \in \mathbb{Z}^{n-m} \times \mathbb{Z}^{q-p}$ is stoichiometric matrix for fast reactions

  Slow variables are typically **total protein concentrations**, fast variables **metabolites and biochemical complexes**

Model reduction using time-scale hierarchy

- Separation of fast and slow variables allows original model to be rewritten as coupled slow and fast subsystems

$$\dot{x}^s = N^s\, v^s(x^s, x^f)$$

$$\dot{x}^f = N^{s'}\, v^s(x^s, x^f) + N^f\, v^f(x^s, x^f) \approx N^f\, v^f(x^s, x^f)$$

- Under **quasi-steady-state approximation (QSSA)**, fast variables are assumed to instantly adapt to slow dynamics

$$\dot{x}^f = 0 \;\Rightarrow\; N^f\, v^f(x^s, x^f) = 0$$

Mathematical basis for QSSA is given by Tikhonov's theorem

Heinrich and Schuster (1996), *The Regulation of Cellular Systems*, Chapman & Hall

Khalil (2001), *Nonlinear Systems*, Prentice Hall, 3rd ed.

Model reduction using time-scale hierarchy

- QSSA implicitly relates steady-state value of fast variables to slow variables

$$x^f = g(x^s),\ g\ :\ \mathbb{R}_+^m \rightarrow \mathbb{R}_+^{n-m}$$

- This gives **reduced model on the slow time-scale**

$$\dot{x}^s = N^s\, v^s(x^s, g(x^s))$$

Reduced model describes direct and indirect dependencies between slow variables (total protein concentrations)

Mathematical representation of effective **gene regulatory network**

- Notice
  - Generally function g is not easy to obtain due to nonlinearities
  - Function g depends on unknown parameter values

Jacobian matrix and regulatory structure

- **Derivation of interaction structure** between slow variables by computation of **Jacobian matrix**

$$\mathcal{J} = \frac{\partial \dot{x}^s}{\partial x^s} = \underbrace{N^s \frac{\partial v^s(x^s, x^f)}{\partial x^s}}_{\text{Direct regulation by transcription factors}} + \underbrace{N^s \frac{\partial v^s(x^s, x^f)}{\partial x^f} \frac{\partial g(x^s)}{\partial x^s}}_{\text{Indirect regulation through metabolism}}$$

- Implicit differentiation of $N^f\, v^f(x^s, x^f) = 0$ yields

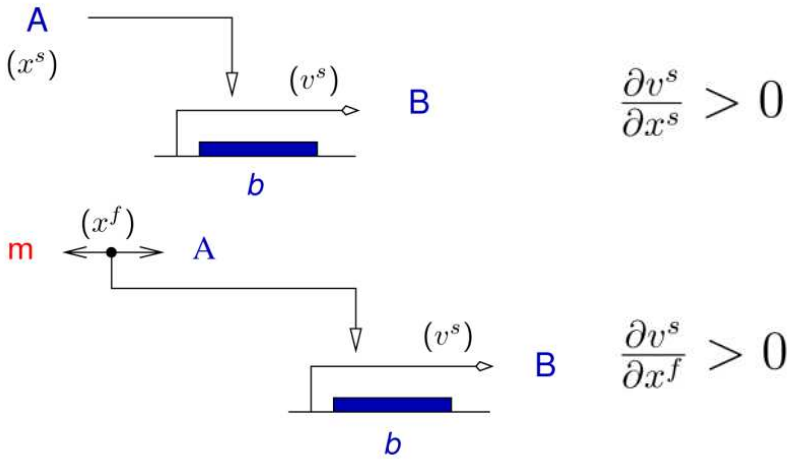
$$\frac{\partial g(x^s)}{\partial x^s} = \underbrace{-M^{-1}\, N^f}_{\text{Concentration control coefficients}} \frac{\partial v^f(x^s, x^f)}{\partial x^s}$$

where $M = N^f\, \partial v^f(x^f, x^s)/\partial x^f$ is Jacobian matrix of fast system

Determination of interaction signs

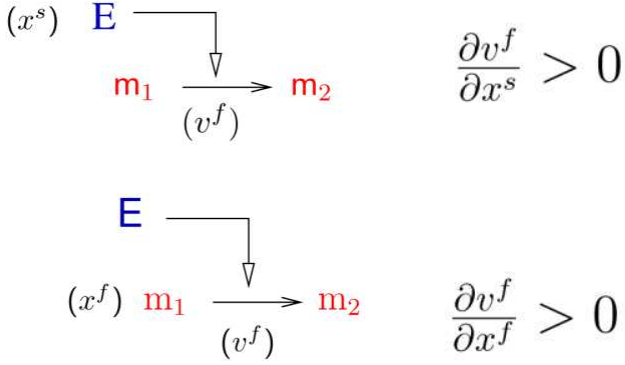
- Can we derive **signs for regulatory interactions** (elements of Jacobian matrix), without knowledge on rate laws and parameter values?
- Idea: exploit fact that **signs of elasticities** are known

  Rate laws are generally monotone functions in variables



$$\frac{\partial v^s}{\partial x^s} > 0$$

$$\frac{\partial v^s}{\partial x^f} > 0$$



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Determination of interaction signs

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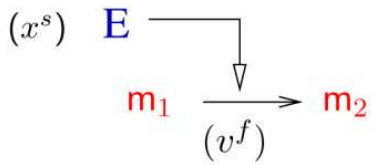
  Rate laws are generally monotone functions in variables
- Notice
  - Reversible reactions: signs of $\partial v^f(x^s, x^f)/\partial x^s$ change with flux direction



$$\frac{\partial v^f}{\partial x^s} > 0$$

Determination of interaction signs

- Resolution of signs of (large) algebraic expressions defining interaction signs by means of computer algebra tools

$$\mathcal{J} = \frac{\partial \dot{x}^s}{\partial x^s} = N^s \frac{\partial v^s(x^s, x^f)}{\partial x^s} + N^s \frac{\partial v^s(x^s, x^f)}{\partial x^f} \frac{\partial g(x^s)}{\partial x^s}$$

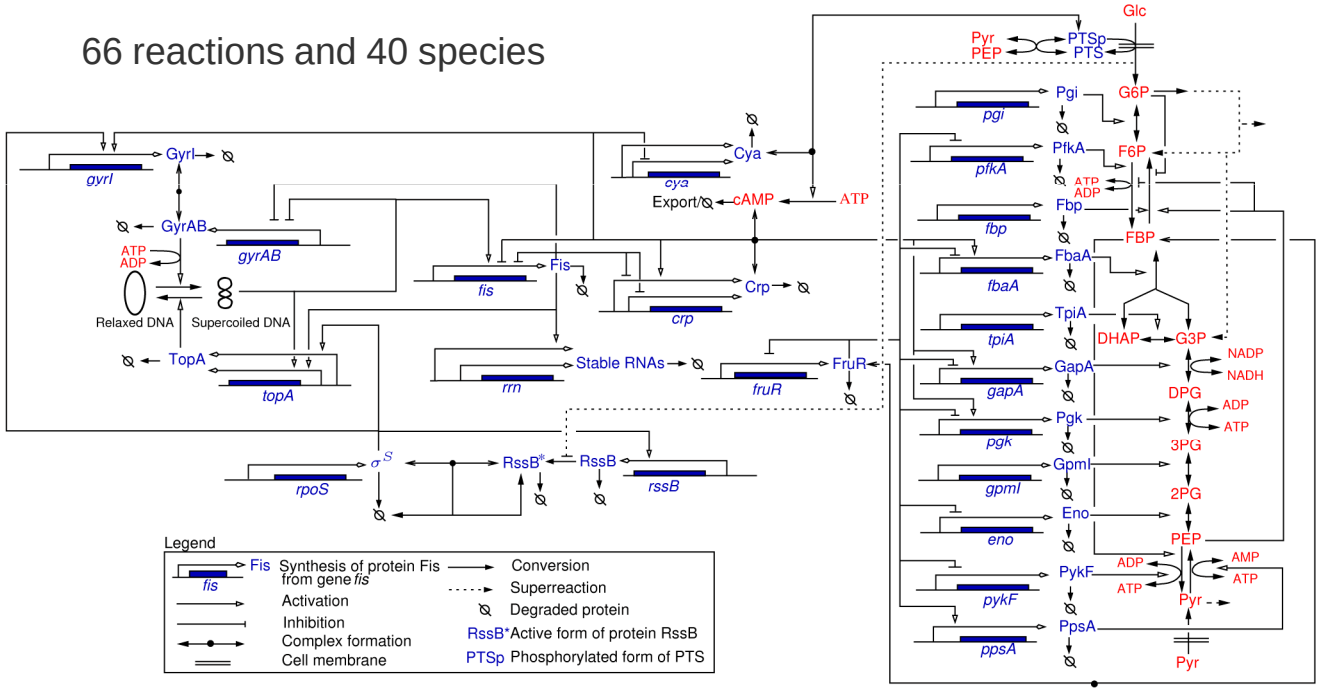
Symbolic Math Toolbox in Matlab

- Use of additional constraints in sign resolution
  - **Stability assumption for fast system**: necessary condition for stability is that coefficients of characteristic polynomial $det(M - \lambda I) = 0$ have same sign
  - **Experimental determination** of some of the signs of concentration control coefficients in $\frac{\partial g(x^s)}{\partial x^s}$ (if available)

Application to *E. coli* carbon assimilation

- Development of model of carbon assimilation network, analysis under following conditions:

  Glycolysis/gluconeogenesis (growth on glucose/pyruvate)



Baldazzi *et al.* (2010), *PLoS Comput. Biol.*, 6(6):e1000812

Application to *E. coli* carbon assimilation

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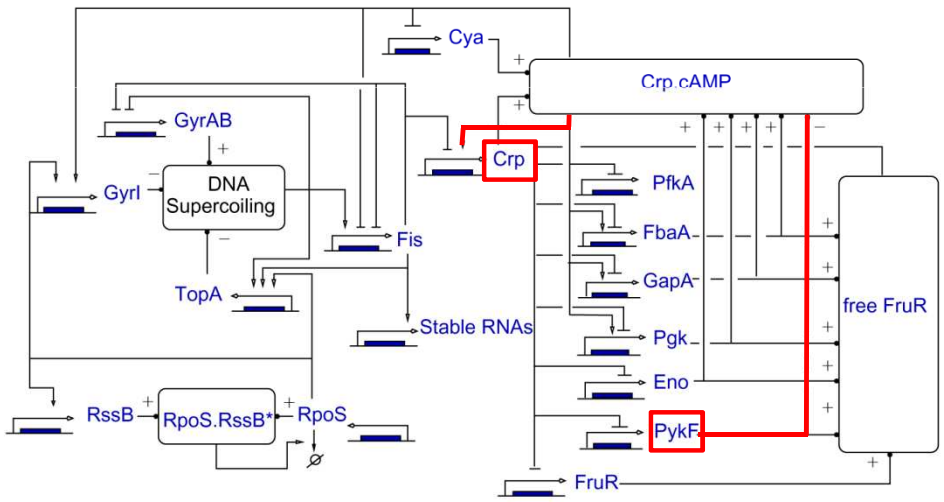
  Glycolysis/gluconeogenesis (growth on glucose/pyruvate)

$$\mathcal{J} = \frac{\partial \dot{x}^s}{\partial x^s}$$



	Regulators															
	PfkA	FbaA	GapA	Pgk	Eno	PykF	Cya	Crp	Fis	GyrAB	Gyrl	TopA	RpoS	RssB	stable RNAs	FruR
pfkA	0	-	-	-	-	-	0	0	0	0	0	0	0	0	0	-
fbaA	0	-/+	-/+	-/+	-/+	-	+	+	0	0	0	0	0	0	0	-
gapA	0	-/+	-/+	-/+	-/+	-	+	+	0	0	0	0	0	0	0	-
pgk	0	-/+	-/+	-/+	-/+	-	+	+	0	0	0	0	0	0	0	-
eno	0	-	-	-	-	-	0	0	0	0	0	0	0	0	0	-
pykF	0	-	-	-	-	-	0	0	0	0	0	0	0	0	0	-
cya	0	-	-	-	-	+	-	-	0	0	0	0	0	0	0	0
crp	0	+	+	+	+	-	+	+	-	0	0	0	0	0	0	0
fis	0	0	0	0	0	0	-	-	-	+	-	-	0	0	0	0
gyrAB	0	0	0	0	0	0	0	0	-	-	+	+	0	0	0	0
gyrI	0	0	0	0	0	0	+	+	0	0	0	0	+	0	0	0
topA	0	0	0	0	0	0	0	0	+	+	-	-	+	0	0	0
rpoS	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0
rssB	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0
rrn	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0
fruR	0	-	-	-	-	-	0	0	0	0	0	0	0	0	0	-

Glycolysis with allosteric effects

- Few fast variables couple metabolism to gene expression

Network is densely connected

- Contrary to what is often maintained, gene regulatory network is found to be **densely connected**
- Strong connectivity arises from indirect interactions mediated by metabolism
  - $\mathcal{M}^0$: transcriptional network consisting of direct interactions only
  - $\mathcal{M}^2_{glyco}$: gene regulatory network in glycolytic growth conditions including direct and indirect interactions

	$\mathcal{M}^0$	$\mathcal{M}^1_{glyco}$	$\mathcal{M}^2_{glyco}$	$\mathcal{M}^1_{neo}$	$\mathcal{M}^2_{neo}$
Number of feedback loops	4	2388	9246	24	2257
Maximal loop length	2	12	12	6	12
Average connectivity	1.4	4.7	5.2	2.8	4.4

- Experimental evidence for indirect interactions

Siddiquee *et al.* (2004), *FEMS Microbiol. Lett.*, 235:25–33

Network is largely sign-determined

- Derived gene regulatory network for carbon assimilation in *E. coli* is largely **sign-determined**

  Signs of interactions do not depend on explicit specification of kinetic rate laws or parameter values, but are structural property of system

Glycolysis with allosteric effects

		Regulators															
		PfkA	FbaA	GapA	Pgk	Eno	PykF	Cya	Crp	Fis	GyrAB	GyrI	TopA	RpoS	RssB	stable RNAs	FruR
Genes	*pfkA*	0	-	-	-	-	-	0	0	0	0	0	0	0	0	0	-
	fbaA	0	-/+	-/+	-/+	-/+	-	+	+	0	0	0	0	0	0	0	-
	gapA	0	-/+	-/+	-/+	-/+	-	+	+	0	0	0	0	0	0	0	-
	pgk	0	-/+	-/+	-/+	-/+	-	+	+	0	0	0	0	0	0	0	-
	eno	0	-	-	-	-	-	0	0	0	0	0	0	0	0	0	-
	pykF	0	-	-	-	-	-	0	0	0	0	0	0	0	0	0	-
	cya	0	-	-	-	-	+	-	-	0	0	0	0	0	0	0	0
	crp	0	+	+	+	+	-	+	+	-	0	0	0	0	0	0	0
	fis	0	0	0	0	0	0	-	-	-	+	-	-	0	0	0	0
	gyrAB	0	0	0	0	0	0	0	0	-	-	+	+	0	0	0	0
	gyrI	0	0	0	0	0	0	+	+	0	0	0	0	+	0	0	0
	topA	0	0	0	0	0	0	0	0	+	+	-	-	+	0	0	0
	rpoS	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0
	rssB	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0
	rrn	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0
	fruR	0	-	-	-	-	-	0	0	0	0	0	0	0	0	0	-

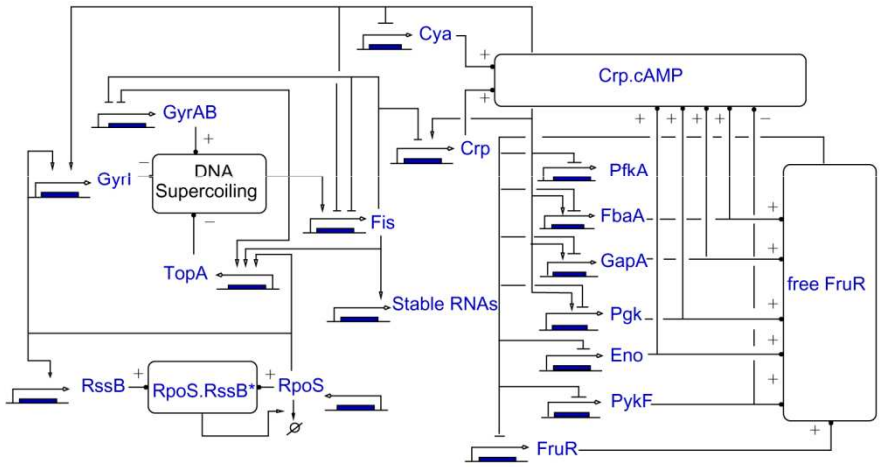
- Sign-determinedness not expected on basis of work in ecology

  Sufficient conditions for sign-determinedness can be formulated using expression for $\mathcal{J}$

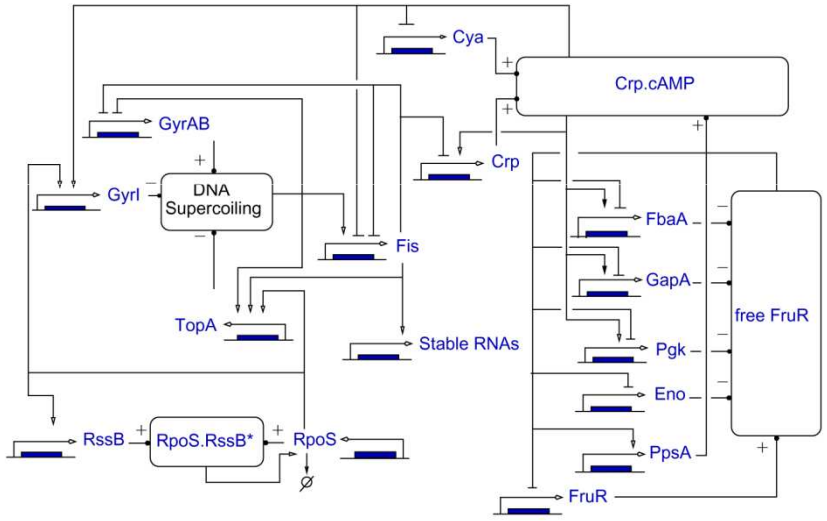
Baldazzi *et al.* (2010), *PLoS Comput. Biol.*, 6(6):e1000812

Interaction signs change with fluxes

- Radical changes in environment may invert signs of indirect interactions, because they change direction of metabolic fluxes and thus signs of elasticities



Network under glycolytic conditions



Network under gluconeogenic conditions

- Dynamic modification of feedback structure in response to environmental perturbations

Key findings and new questions

- Systematic derivation of effective structure of gene regulatory network on time-scale of gene expression

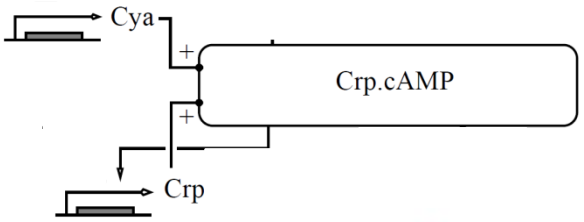
  Weak assumptions on time-scale hierarchies and stability

- Obtained network is at the same time **robust** and **flexible**
  - Robust to changes of kinetic properties (results not dependent on parameter values and rate laws)
  - Flexible rewiring of network structure following radical changes in environment (changes in flux directions)

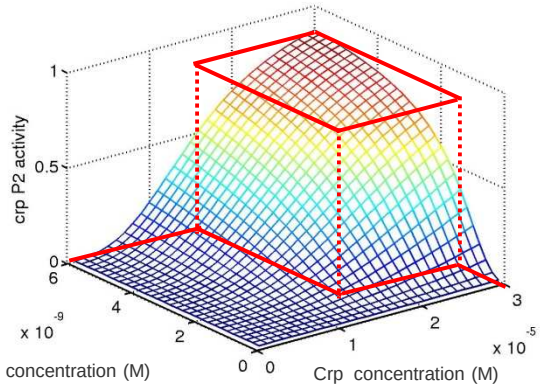
- Results on *E. coli* network raise several issues:
  - To which extent do observations carry over to other regulatory systems in bacteria and higher organisms?
  - How do indirect interactions affect dynamics of networks?

Dynamical analysis of networks

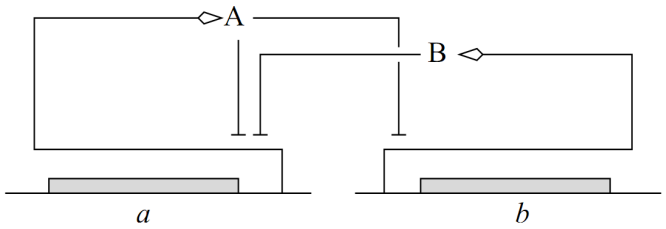
- Reduced networks describe direct and indirect regulatory interactions between genes
- Qualitative dynamics of gene regulatory interactions can be described by **PL models** Glass and Kauffman (1973), *J. Theor. Biol.*, 39(1):103-29
- Translation of network diagrams into PL models
  - Straightforward for direct interactions…
  - … but also possible for indirect interactions



$$\dot{x}_y = \kappa_y^1 + \kappa_y^2 - \gamma_y\, x_y$$
$$\dot{x}_c = \kappa_c^1 + \kappa_c^2\, s^+(x_c, \theta_c^1)\, s^+(x_y, \theta_y^1) - \gamma_c\, x_c$$

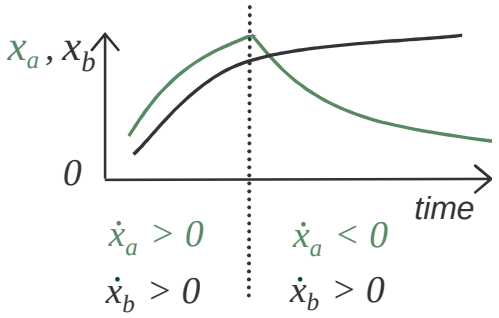


Qualitative analysis of PL models



$$\dot{x}_a = \kappa_a \, s^-(x_a, \theta_{a2}) \, s^-(x_b, \theta_b) - \gamma_a \, x_a$$
$$\dot{x}_b = \kappa_b \, s^-(x_a, \theta_{a1}) - \gamma_b \, x_b$$

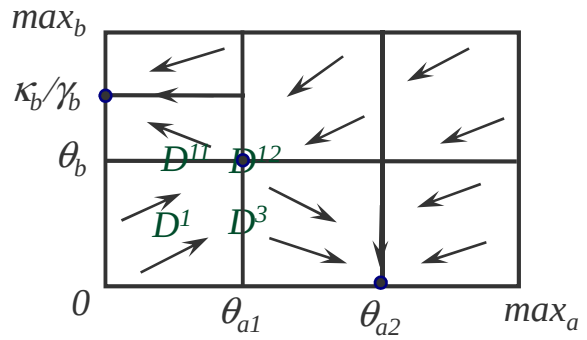
PL models using step functions



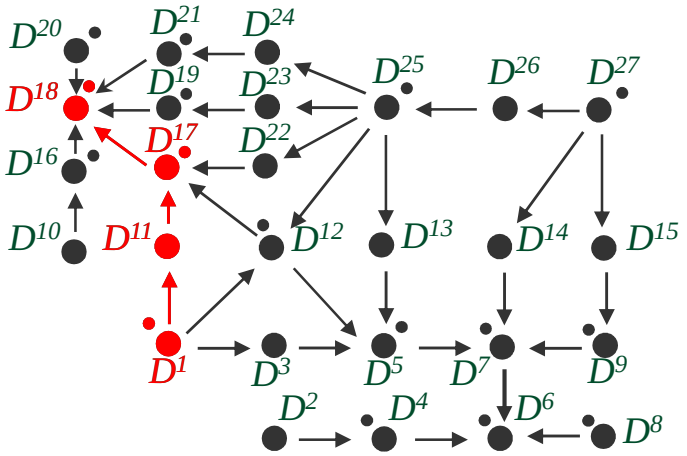
Model-checking for verification of system properties

de Jong et al. (2004), Bull. Math. Biol., 66(2):301-40

Batt et al. (2007), Automatica, 44(4):982-89



Models easy to analyze, using inequalities



D^1: $\dot{x}_a > 0$, $\dot{x}_b > 0$

D^{17}: $\dot{x}_a < 0$, $\dot{x}_b > 0$

D^{18}: $\dot{x}_a = 0$, $\dot{x}_b = 0$

Predictions of qualitative dynamics, robust for large variations in parameter values

Development of PL model

- PL models for growth on glucose and growth on acetate
  - Models have13 equations and require specification of few dozen parameter inequalities
- Calibration of PL models by means of qualitative data
  - Literature data
  - Extension of previous model of network of global regulators

    Ropers *et al.* (2006), *Biosystems*, 84(2):124-152; Ropers *et al.* (2009), in press
  - Hypotheses (educated guesses)

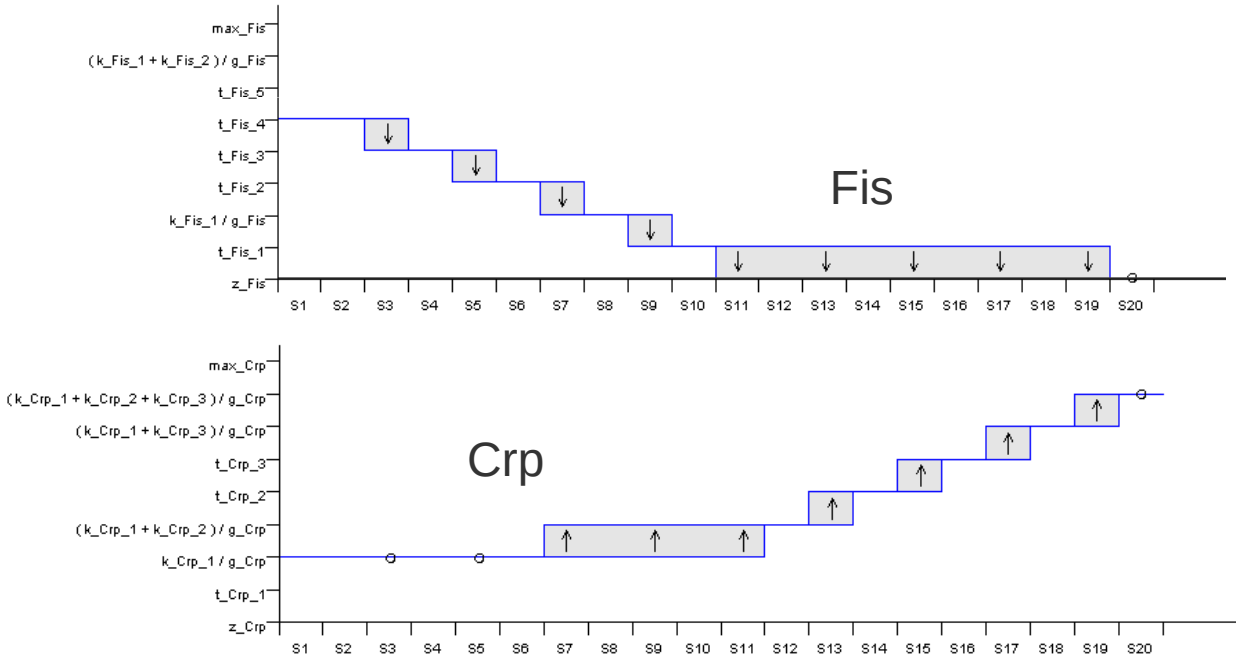
Aim of analysis of PL model

- **Aims of analysis** of network dynamics:
  - Predict evolution of gene expression levels after diauxic shift
  - Study role of indirect interactions mediated by metabolism
- Modeling of **batch experiments**
  - Grow bacteria in M9 with glucose to steady state (glycolysis)
  - Continue growth on (excreted) acetate after glucose exhaustion (gluconeogenesis)

Brice Enjalbert, personal communication

Some preliminary results

- Cross-inhibition between Fis and Crp predicted to play central role in adaptation of gene expression upon glucose depletion
- Predicted gene expression profiles verified by means of reporter gene measurements



Towards quantitative models?

- Above approach leads to models that view metabolism as intermediary between gene regulatory interactions
- However, metabolism is not explicitly modeled

  PL models aggregate and approximate complex rate functions in reduced model

$$\dot{x}^s = N^s \, v^s(x^s, g(x^s)) \quad \Rightarrow \quad \dot{x}^s = f(x^s)$$

- Moreover, models provide qualitative instead of quantitative picture of dynamics

  Qualitative models help provide intuitive idea of global system dynamics, but for some questions quantitative precision is required

Towards quantitative models?

- Another approach explicitly models metabolism and gene expression, followed by integration of two parts

$$N^f\, v^f(x^s, x^f) = 0 \;\Rightarrow\; x^f = g(x^s)$$
$$\dot{x}^s = N^s\, v^s(x^s, x^f)$$

- Approach based on suitable approximations of g
  - Approximations should provide good phenomenological description of metabolic rate laws
  - Minimal number of parameters to facilitate identification of parameter values from experimental data

4. Metabolic model

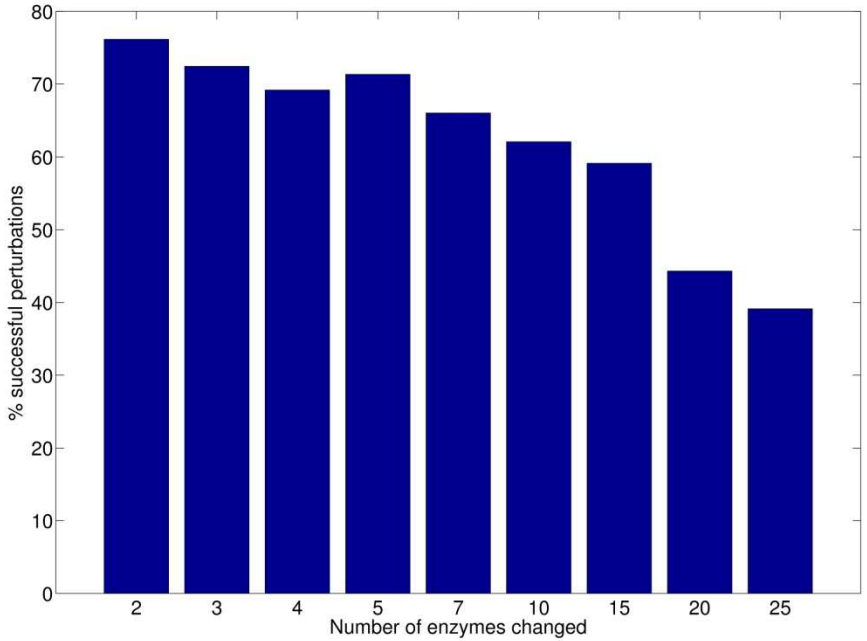
- Toy model entirely specified with ODEs
- 'Experimental' object used to test the quality of various reductions and approximations by comparison of simplified models with complete ODE model
- A suitable approximation would ideally allow us to calculate $g(x^s)$ analytically

Which approximations?

- Various types of linearisation of metabolic effects
- Compare reduced / approximated models with complete ODE-specified model

Assessing approximations of metabolism

- Randomly change enzyme concentrations in a 25-fold range on benchmark model (Matteo Brilli)
- Test steady-state obtention



Approximation 1, from MCT

$$\mathbf{v} = diag\ \boldsymbol{f}(\mathbf{x}) \cdot \mathbf{e}$$

$$d\mathbf{X}/d\mathbf{e} = \Gamma \cdot d\mathbf{v}/d\mathbf{e}$$

$$\Delta \ln \mathbf{X} \sim (diag\ \mathbf{X}_0)^{-1} \cdot \Gamma \cdot diag\ \boldsymbol{f}(\mathbf{X}_0) \cdot \Delta\mathbf{e}$$

Linearization around steady-state using control coefficients

Approximation 2, linlog

Linearization of kinetic laws:

$$\mathbf{v}(\mathbf{x}) \sim \mathit{diag}\ \mathbf{e} \cdot (\mathbf{A} + \mathbf{B} \cdot \ln\mathbf{x})$$

Steady-state implies:

$$\mathbf{N} \cdot \mathbf{v}(\mathbf{X}) = \mathbf{0}$$

$$\ln\mathbf{X} \sim - (\mathbf{N} \cdot \mathit{diag}\ \mathbf{e} \cdot \mathbf{B})^{-1} \cdot \mathbf{N} \cdot \mathit{diag}\ \mathbf{e} \cdot \mathbf{A}$$

Approximation 3, hyperbolic

Suggested from earlier work by Kacser:

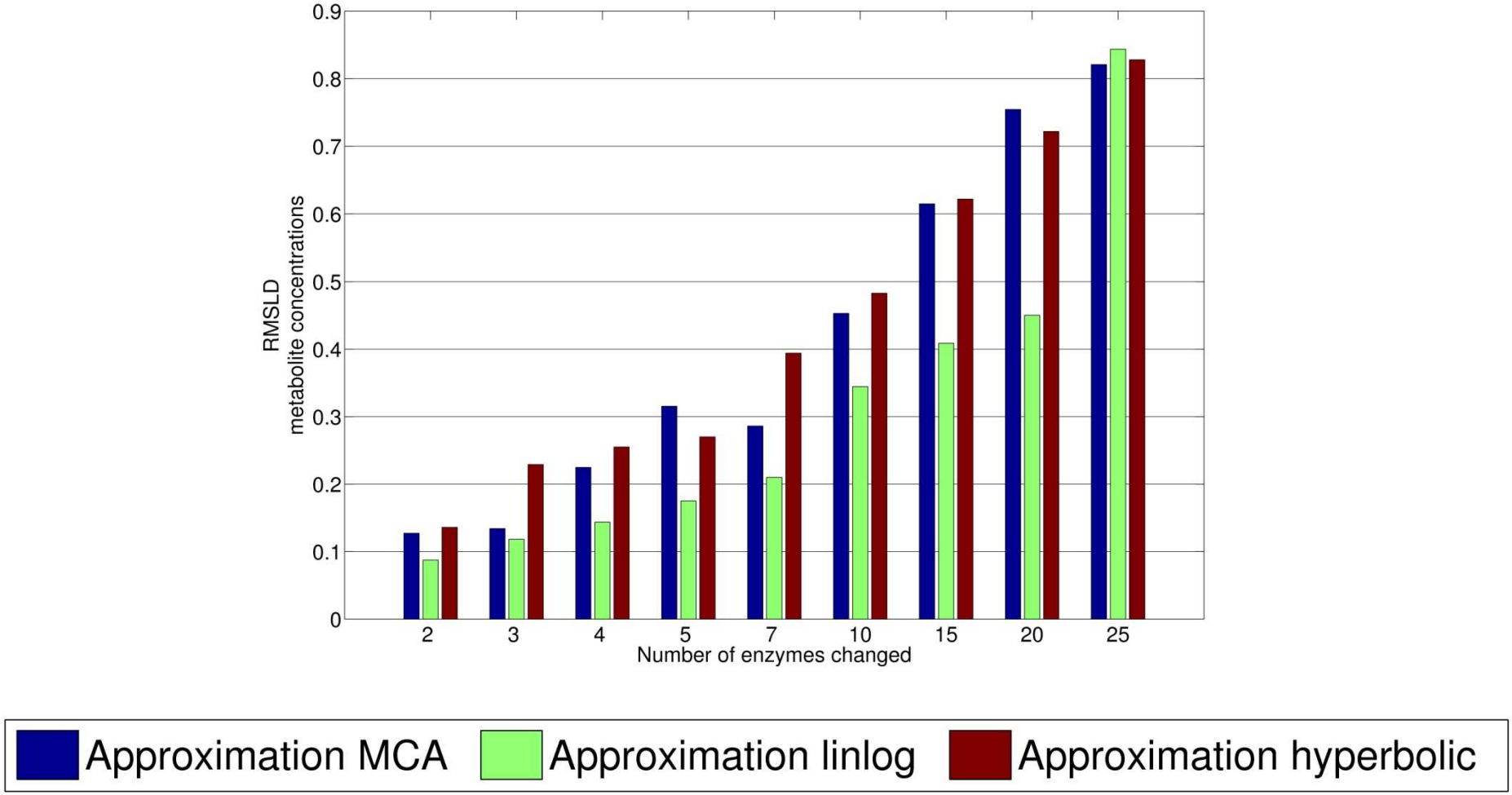
$$\Delta(1/\mathbf{X}) \sim (diag\ \mathbf{X}_0)^{-1} \cdot \mathbf{C}^X \cdot diag\ \mathbf{e}_0 \cdot \Delta(1/\mathbf{e})$$

$$\Delta(1/\mathbf{J}) \sim (diag\ \mathbf{J}_0)^{-1} \cdot \mathbf{C}^J \cdot diag\ \mathbf{e}_0 \cdot \Delta(1/\mathbf{e})$$

Linearization around steady-state using control coefficients

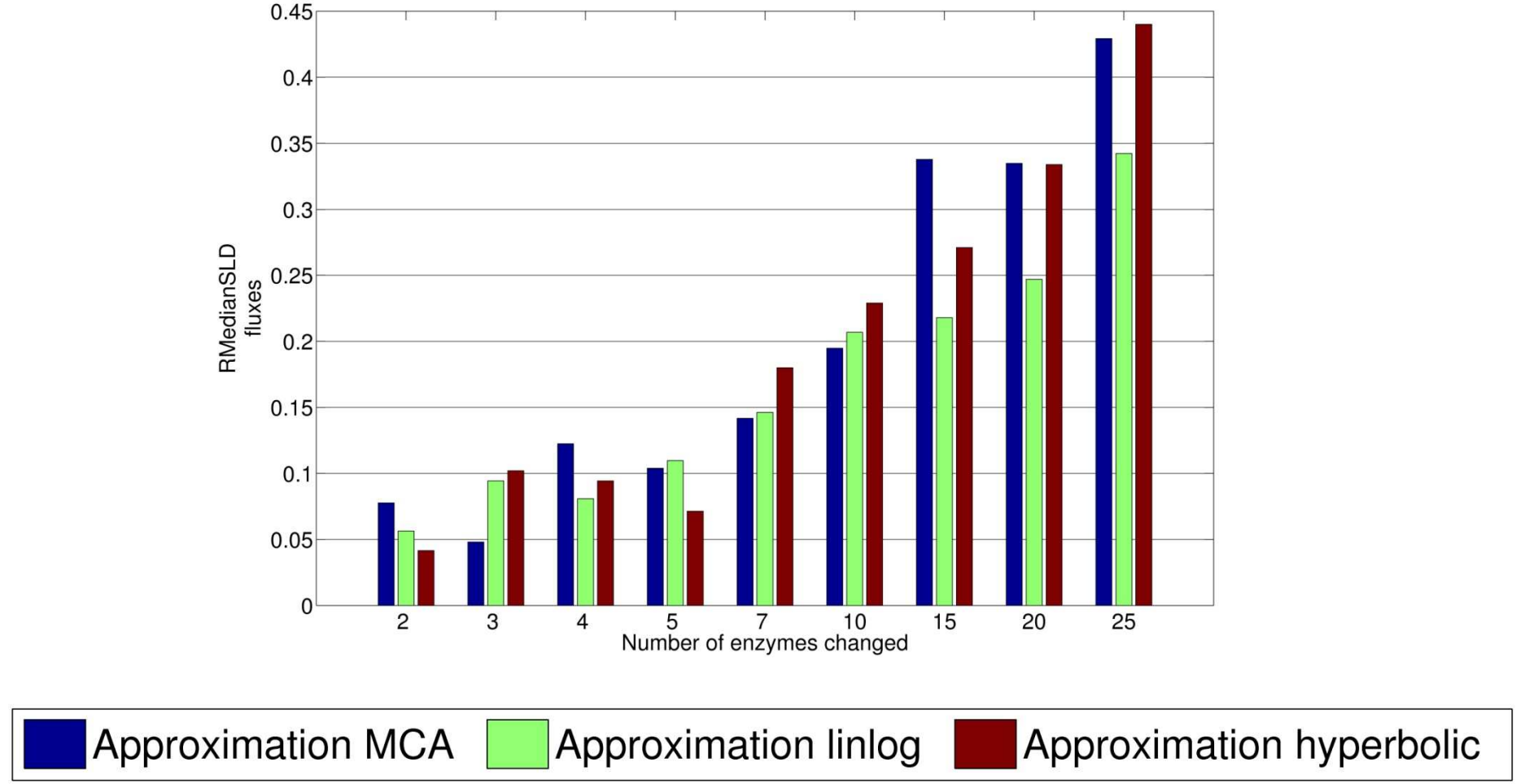
Metabolite estimates

Root mean square Log deviation



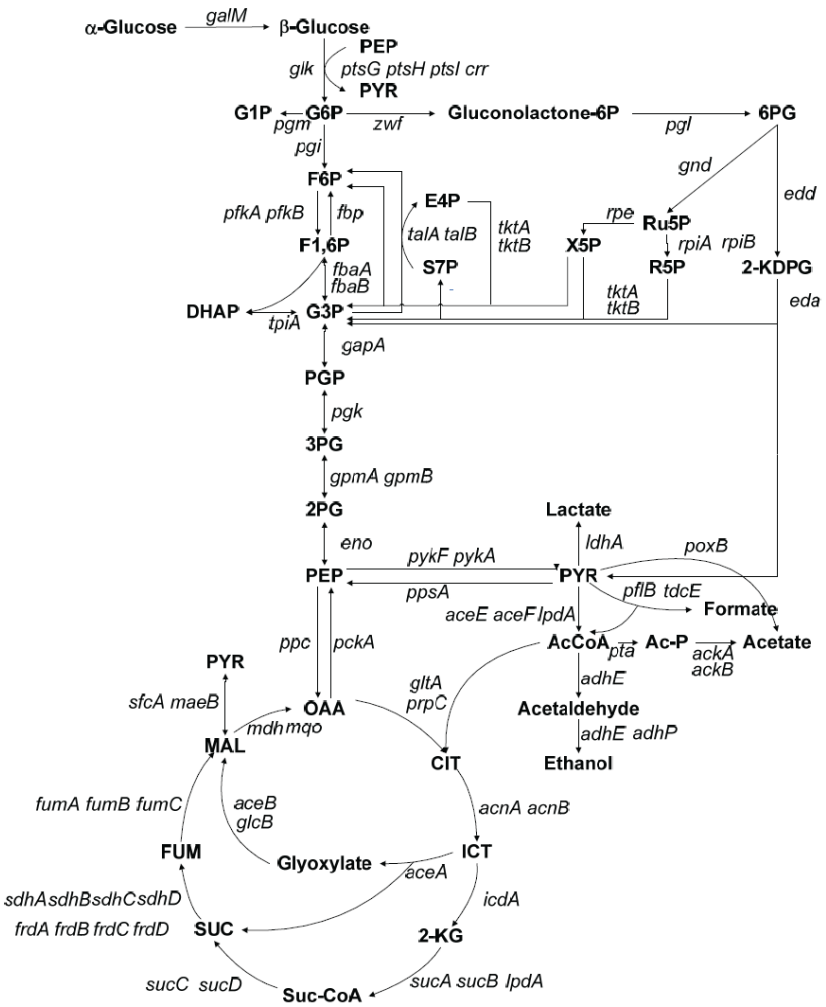
Flux estimates

Median flux absolute Log deviation



Model of *E. coli* carbon metabolism

- Simplified model
  - 32 reactions
  - 17 metabolites
- Linlog approximation
  $\mathbf{J} \sim \mathit{diag}\ \mathbf{e} \cdot (\mathbf{A} + \mathbf{B} \cdot \ln \mathbf{X})$
- → Independent linear regression possible for each reaction if sufficient data available :
  - Fluxes
  - Enzyme expression
  - Metabolite concentrations



Issues with metabolic model identification

- Difficulties to obtain high quality complete datasets (fluxes, metabolite and enzyme concentrations) with sufficient numbers of distinct observations
- Missing data can be handled efficiently by EM or maximum likelihood methods (Berthoumieux *et al.*, submitted)
- Identifiability issues arise when there is insufficient variability or dependencies between metabolite concentrations because of
  - Reactions close to equilibrium
  - Steady-state constraints
  - Homeostasis

  → Usefulness of dynamic non steady-state measurements (difficult to obtain)

Working around identifiability issues

- Use Principal Component Analysis on ln**X**
  - Reduce metabolite data by Singular Value Decomposition

$$\ln \mathbf{X} - \overline{\ln \mathbf{X}} = \mathbf{U}\mathbf{S}\mathbf{V}^T$$

  - Determine effective dimension of ln**X** from singular values σ_i, neglecting σ_i^2 smaller than experimental variance
  - Reduce metabolite data and reformulate identification accordingly

$$\mathbf{Y} = \mathbf{U}_r^T \ln \mathbf{X}$$

  - Estimate parameters $\mathbf{B}_r$ for the reduced model such that

$$\mathbf{J} / \mathbf{e} - \overline{\mathbf{J} / \mathbf{e}} = \mathbf{B} \ln \mathbf{X} = \mathbf{B}_r \mathbf{Y}$$

  - One among an infinite number of choices for full parameters is $\mathbf{B} = \mathbf{B}_r \mathbf{U}_r^T$

5. Integrating gene and metabolic models

- Identify separately the fast component (metabolic) and the slow component (gene expression)
- Use the resulting analytical model of metabolic steady-states as a ‘plugin’ function in the gene network model

$$N^f \, v^f(x^s, x^f) = 0 \;\Rightarrow\; x^f = g(x^s)$$
$$\dot{x}^s = N^s \, v^s(x^s, x^f)$$

- Critical issue: identification methods and, especially, quality and quantity of experimental data

Experimental data on metabolism

- Quantification of extra-cellular metabolites by means of nuclear magnetic resonance (NMR) spectroscopy

Brice Enjalbert, personal communication

Experimental data on metabolism

- Quantification of intra-cellular metabolites by means of mass spectrometry

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Isotope Dilution Mass Spectrometry

Experimental data on metabolism

- Quantification of intra-cellular metabolites by means of mass spectrometry

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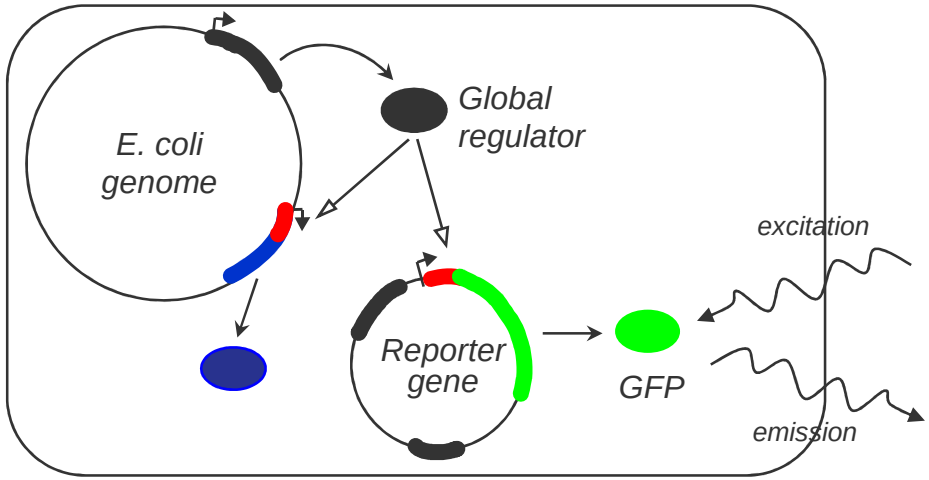
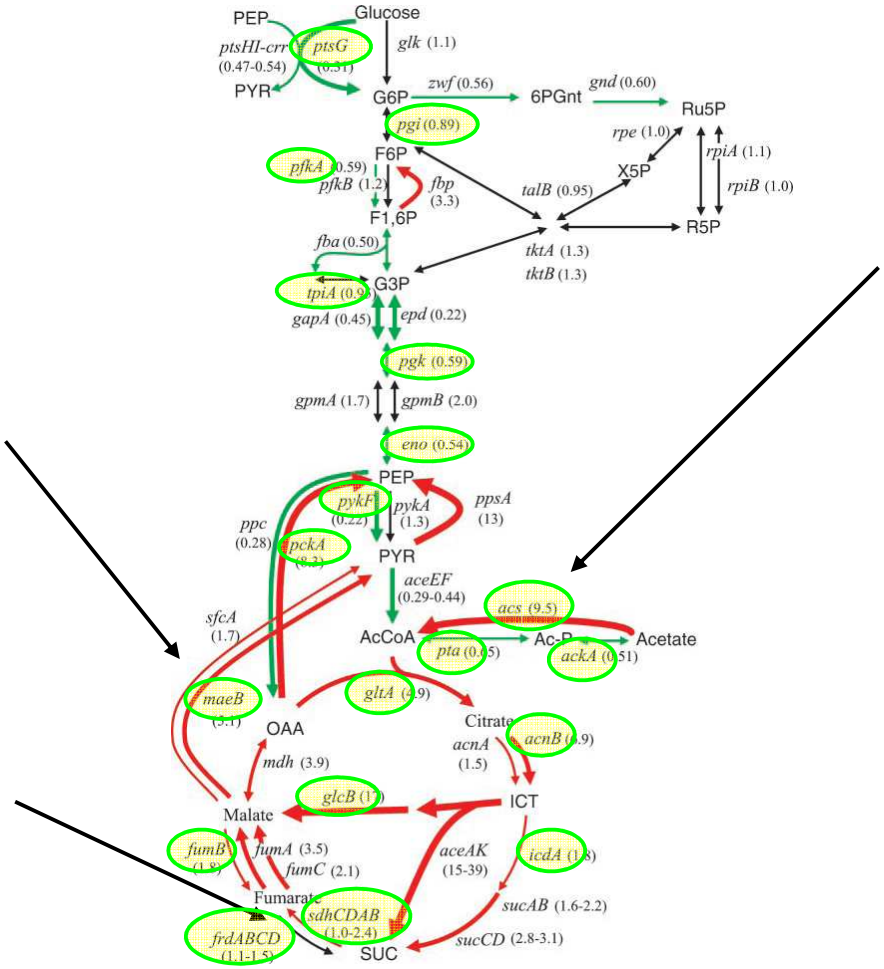
Experimental data on gene expression

- Quantification of gene expression by means of fluorescent and luminescent reporter genes
  - Expression of reporter gene is proportional to expression of target gene



Experimental data on gene expression



Hans Geiselmann, personal communication

Prospect:
Roles of metabolic and gene regulation

- Identify parameters of the reduced system from data
- Study the metabolic response in the model when gene regulation is abolished
- Evaluate (quantify) the contribution of gene regulation to the metabolic response
- Conversely calculate the contribution of metabolic effects to gene regulation
- Understand the biological rationale underlying the distribution of regulation between metabolism and gene expression